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Phylogenetic affinity and taxonomic reassessment of *Pseudoidium kalanchoes*

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Abstract—*Pseudoidium kalanchoes*, retrieved from powdery mildew on *Kalanchoe* spp. in Asia, has been re-examined and reassessed based on phylogenetic analyses of the ITS and 28S rDNA regions. The results confirmed that sequences retrieved from Asian *Kalanchoe* powdery mildew have 100% congruence with sequences obtained from *Erysiphe sedi* on *Sedum* spp. A German collection of *P. kalanchoes* on *Kalanchoe blossfeldiana* was morphologically and genetically examined and analyzed and is designated as neotype with an ex-neotype reference sequence. Morphologically, the asexual morph on *Kalanchoe* spp. agrees with the asexual morph of *E. sedi*. Consequently, *P. kalanchoes* is reduced to synonymy with *E. sedi*.

Key words —*Erysiphales*, "*Oidium calanchoeae*," phylogeny

Introduction

Lüstner (1935) introduced the name "*Oidium calanchoeae*" for an asexual powdery mildew on *Kalanchoe* sp. (very probably *K. blossfeldiana* Poelln.) from Germany. He failed to present the Latin description or diagnosis required by ICN (Shenzhen) Art. 39.1, making his binomial invalid; and his epithet "*calanchoeae*" required a twofold correction, to *kalanchoes*, an orthography based on the accepted name of the host genus (ICN Art. F.9.1), *Kalanchoe* Adans. 1763 (instead of *Calanchoe* Pers. 1805), and use of the correct genitive case ending (ICN Art. 32.2). Braun (1987: 602) validated

Oidium kalanchoes by adding a Latin description and an effective typification based on Lüstner's original material (although this could not be traced). The corrected orthography was first published in Braun & al. (2003), and Braun & Cook (2012) later combined this species in *Pseudoidium* Y.S. Paul & J.N. Kapoor.

Pseudoidium kalanchoes has been reported (sometimes under synonyms or misdeterminations) from Asia, Australasia, Europe, and North America on crassulacean hosts: *Crassula ovata, Kalanchoe blossfeldiana, K. (Bryophyllum) daigremontiana, K. (Bryophyllum) delagoensis, K. (Bryophyllum) pinnata, Kalanchoe* spp., and *Sedum praealtum* (Adhikari 2017; Amano 1986; Boesewinkel 1980; Braun 1987, 1995; Braun & Cook 2012; Braun & al. 2003; Cho & al. 2012; Fang & al. 2017; Ialongo 1992; Jage & al. 2010; Liu 2010; Nagy 1975; Tang & al. 2016; see also Table 1). Chinese collections on *K. blossfeldiana* and *K. pinnata* were assigned to *Erysiphe sedi* on the basis of ITS analyses (Tang & al. 2016; Fang & al. 2017). The three hosts classified in *Kalanchoe* sect. *Bryophyllum* are retained in *Kalanchoe* (rather than in genus *Bryophyllum*) on the basis of phylogenetic analyses (Gehrig & al. 2001, Mort & al. 2010).

The present examinations aimed at re-examining and analyzing German collections through morphological and molecular methods in order to clarify the identity of *Oidium kalanchoes* and to stabilize the application of this name by neotypification with an ex-neotype reference sequence.

Materials & methods

Collection and maintenance of powdery mildew isolates

The powdery mildew isolate used for sequence analyses was collected from naturally infected *Kalanchoe blossfeldiana* plants from Germany in January 2018.

Morphological characterization and molecular analysis described below were performed using the single spore isolate JKI-GFZ-C20-EM25A, obtained by transferring a single conidium onto washed cut-off *Kalanchoe* leaves. Inoculated cuttings were incubated in plastic boxes at room temperature and natural day–night periods for two to three weeks until colonies could be detected. To ensure a pure isolate, four subsequent sub-cultures were performed. Uninoculated leaves served as controls. Artificially infected leaves were deposited at the Herbarium of the Institute of Biology, Department of Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Germany (HAL) as voucher HAL 3298 F.

Morphological characterization

For morphological characterization, fresh powdery mildew structures were stripped off the leaf with sticky tape and mounted in water. Herbarium samples were mounted in lactic acid and gently heated. Mycelia from the upper and lower leaf surfaces were inspected. Morphological characteristics covering size and shape of conidia (n ≥100) and conidiophores (n ≥50), position of the basal septum, shape and position of hyphal appressoria, and presence or absence of fibrosin bodies were assessed. Conidial length and width were analyzed following Frank (1990) with modifications: the applied values indicate the minimum, lower limit, upper limit, and maximum values; lower and upper limits indicate the range of 90% of all values (not 95% as described by Frank 1990).

All morphological characteristics were examined using a Zeiss® Axio Imager.A1 standard light microscope equipped with an AxioCam MRc5 camera and differential interference contrast at 200×, 400×, and 1000×. Measurements were made and images were taken with the calibrated Axiovision software rel. 4.8, and the images were processed using Adobe® Photoshop CS4 software version 11.0.

Conidial germination patterns were assessed following the method of Zaracovitis (1965) with slight modifications. Preferably young conidia (~ 24 h) were dusted onto microscope slides and incubated in a moist chamber at 20 ± 1 °C in the dark for 24–48 h and analysed and documented as above. The patterns were compared with those described in Cook & Braun (2009).

Molecular characterization

Total DNA was extracted from conidia and mycelia using the Qiagen DNeasy plant mini kit following the manufacturer's instructions with slight modifications. The internal transcribed spacer (ITS) was amplified using the primers ITS5 and PM6 (Takamatsu & Kano 2001), and the 28S rRNA was amplified using PM5 (Takamatsu & Kano 2001) and NLP2 (Hirose & al. 2005). The reaction mix contained Hot FirePol Mastermix (containing 7.5 mM $MgCl_2$), 0.2 µM of each primer, and 4 µl template DNA in a total volume of 50 µl. PCR was performed in a MyCycler thermal cycler with an initial denaturation step at 95 °C for 13 min; followed by 40 cycles of 30 s at 94 °C for denaturation, 30 s/50 s (ITS/28S rRNA) at 52 °C for annealing, and 30 s/50 s (ITS/28S rRNA) at 72 °C for extension; and a final extension for 10 min at 72 °C. PCR products were purified using a MSB Spin PCRapace Kit. Fragments were bidirectionally sequenced twice with the same primers used for PCR by LGC Genomics GmbH. A contig was generated and edited using CLC Main Workbench 8.1 following the EPPO recommendations for sequence analysis (OEPP/EPPO 2016). The consensus sequence was deposited in GenBank under the accession number MK411006.

The sequence obtained for the isolate JKI-GFZ-C20-EM25A was aligned to sequences of three *Erysiphe sedi* isolates and 12 additional isolates from the *Erysiphe aquilegia* clade (see Takamatsu & al. 2015 for further details of the sequences concerned) spanning the same region using CLC Main Workbench 8.1 with the settings gap open cost 10.0 and gap extension cost 1.0 and the very accurate alignment mode. *Erysiphe linderae* (LC010067; Abasova & al. 2018) was chosen as outgroup taxon. The alignment was manually refined using CLC Main Workbench.

A phylogenetic tree was constructed using the Neighbor-Joining method and the nucleotide substitution model Kimura´s (1980) two parameter model with a transition/transversion ratio of 2.0 (Fig. 1). The strength of the internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (CLC Main Workbench 8.1).

Results

Phylogenetic analysis

Sequence data including genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA (partial and complete) obtained from the isolate JKI-GFZ-C20-EM25A (MK411006) showed 100% identity to three sequences of *E. sedi* (LC010045, LC010046, LC010047) spanning the same region deposited in GenBank (Fig. 1). They were also identical to sequences of *E. hommae* (LC009926) and *Pseudoidium hortensiae* (LC009915). All sequences belong to the *Erysiphe aquilegiae* clade (see Takamatsu & al. 2015), which is characterized by insufficient resolution on species level, indicating that the resolution of the region analyzed is not high enough to distinguish all members of this clade. However, both morphological characteristics and host range support assignment of JKI-GFZ-C20-EM25A to *E. sedi*.

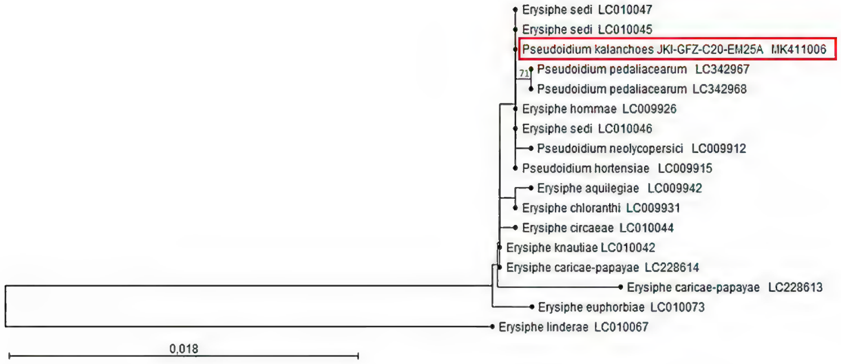
Erysiphe sedi LC010047
Erysiphe sedi LC010045
Pseudoidium kalanchoes JKI-GFZ-C20-EM25A MK411006
71
Pseudoidium pedaliacearum LC342967
Pseudoidium pedaliacearum LC342968
Erysiphe hommae LC009926
Erysiphe sedi LC010046
Pseudoidium neolycopersici LC009912
Pseudoidium hortensiae LC009915
Erysiphe aquilegiae LC009942
Erysiphe chloranthi LC009931
Erysiphe circaeae LC010044
Erysiphe knautiae LC010042
Erysiphe caricae-papayae LC228614
Erysiphe caricae-papayae LC228613
Erysiphe euphorbiae LC010073
Erysiphe linderae LC010067
0,018

Fig. 1. Phylogenetic analysis (combined data of ITS1, 5.8S rRNA, ITS2, and 28S rRNA complete and partial sequences) for the isolate JKI-GFZ-C20-EM25A (framed in red) including 15 selected sequences of the *Erysiphe aquilegiae* clade. The bootstrap support value for maximum likelihood >70% is presented above the node. The tree is rooted to *Erysiphe linderae* (LC010067).

Morphology

The following description is based on collections on *Kalanchoe* spp. (Eliade 1990, Bolay 2005, Liu 2010, Braun & Cook 2012, present examinations):

Erysiphe sedi U. Braun, Feddes Repert. 92(7–8): 502, Sept. 1981. Fig. 2

= *Erysiphe sedi* R.Y. Zheng & G.Q. Chen, Sydowia 34: 253, 31 Dec. 1981, nom. illeg.

= *Oidium kalanchoes* Lüstner ex U. Braun, Beih. Nova Hedwigia 89: 602, 1987, as "*calanchoeae*" [validation of "*Oidium calanchoeae*" Lüstner, Nachrichtenbl. Deutsch. Pflanzenschutzdienst 15(4): 41, 1935, nom. inval., ICN (Shenzhen) Art. 39.1]. Type: Germany, on leaves of *Kalanchoe blossfeldiana*, 1935, G. Lüstner (**holotype; not preserved**). Germany, Niedersachsen, Braunschweig, Julius Kühn Institute, greenhouse, on living leaves of *Kalanchoe blossfeldiana*, Jan. 2018, M. Götz (**neotype,** HAL 3298 F, **designated here** MBT 385509; GenBank MK411006.)

≡ *Pseudoidium kalanchoes* (Lüstner ex U. Braun) U. Braun & R.T.A. Cook, Taxon. Man. *Erysiphales*: 608, 2012.

Mycelium amphigenous, also caulicolous, forming white or grayish white patches, sometimes confluent or effuse, persistent. Hyphae straight to flexuous-sinuous, branched, (2–)4–8(–10) μm wide, septate, hyaline, thin-walled, smooth. Hyphal appressoria solitary or in opposite pairs, 1–4 per cell, almost nipple-shaped to distinctly lobed, 5–10 μm diam. Conidiophores arising from the upper surface of mother cells, erect, 40–200 μm long. Foot-cells cylindrical, straight, (15–)18–50(–60) × 6–11 μm, followed by (1–)2(–3) cells, usually about as long as the foot-cell, shorter or even longer, occasionally followed by a very long cell up to 90 μm, forming conidia singly. Conidia cylindrical to ellipsoid, (22–)30–55(–85) × (10.5–)14–22(–25) μm, length/width ratio (1.5–)1.7–3.2(–3.8). Germ Tubes *Pseudoidium* type, occasionally in a longitubus pattern, usually with a single perihilar germ tube, rarely with 2–3 germ tubes, usually short to moderately long, straight, unbranched and aseptate, occasionally with a single septum, very rarely branched, apex usually with a lobed appressorium, sometimes only swollen and unlobed.

Specimens examined—GERMANY. Hessen: sine loco: on living leaves of *Kalanchoe blossfeldiana*, 1.IV.1998, J. Dalchow (HAL 405 F); Wiesbaden, on living leaves of *Crassula ovata*, 12.V.2003, S. Krause (HAL, s.n.). Niedersachsen: Braunschweig, on living leaves of *Crassula ovata*, I.2003, U. Brielmaier-Liebetanz (HAL, s.n.); Braunschweig, Julius Kühn Institute, greenhouse, on living leaves of *Kalanchoe blossfeldiana*, I.2018, M. Götz (HAL 3298 F, neotype).

Discussion

Until the application of molecular methods for the purpose of identification of powdery mildew species, the names *Pseudoidium/Oidium kalanchoes* were widely used for an asexual morph occurring on *Kalanchoe blossfeldiana* and other *Kalanchoe* species (including *Bryophyllum*) as well as *Crassula ovata* (Eliade 1990, Braun 1995, Braun & al. 2003, Bolay 2005, Liu 2010, Braun & Cook 2012). First molecular

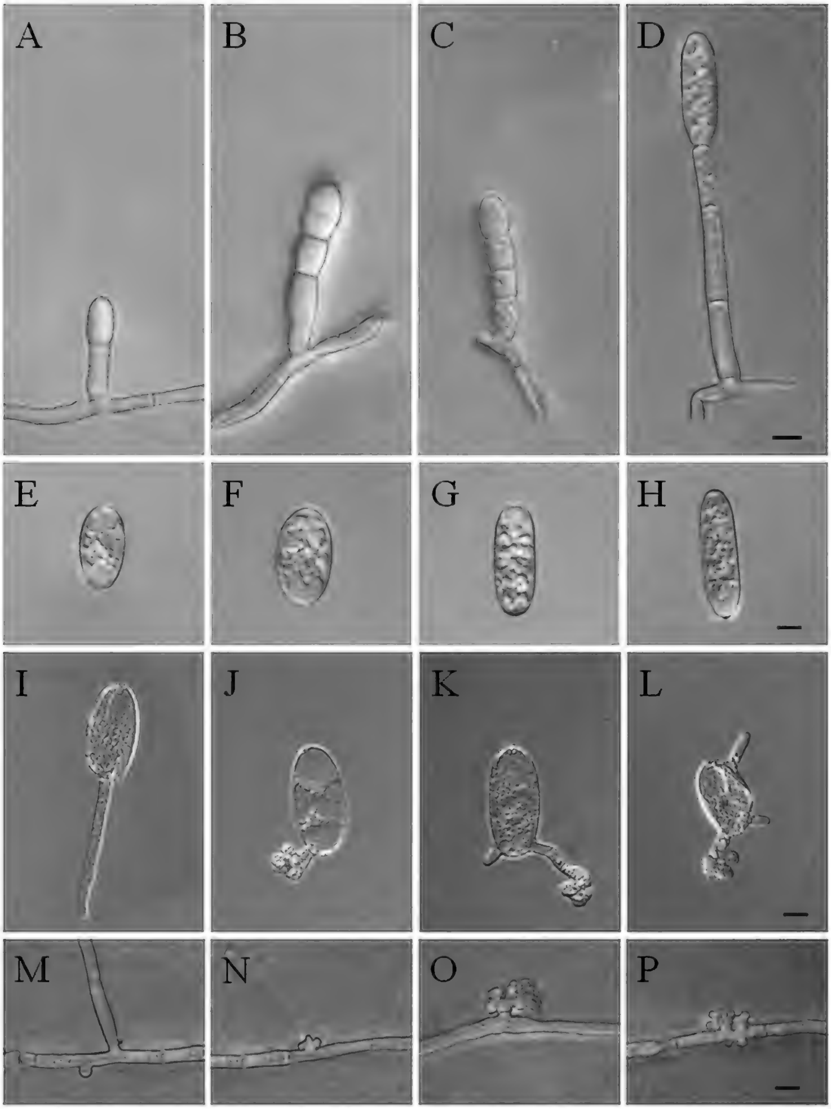

Fig. 2. *Pseudoidium kalanchoes* on *Kalanchoe blossfeldiana* (neotype, HAL 3298 F): A–D. Conidiophores; E–H. Conidia; I–L. Conidial germination types in vitro [I. Longitubus pattern; J–L. *Pseudoidium* type]; M–P. Hyphal appressoria (background removed with Adobe Photoshop to clarify relevant features of the conidiophores). Scale bars = 10 µm.

examinations of this powdery mildew in Asia on *Kalanchoe* spp. raised serious doubts as to the taxonomic status and true affinity of this anamorph-typified powdery mildew species (Cho & al. 2012, Tang & al. 2016, Fang & al. 2017). On the basis of analyses of ITS data and corresponding morphological traits, these authors identified Chinese and Korean powdery mildew specimens on *Kalanchoe* spp. as *Erysiphe sedi*. Therefore, it was logical to assume that *P. kalanchoes* must be considered a heterotypic synonym of *E. sedi*. In order to confirm this assumption, it was necessary to neotypify *P. kalanchoes* (type not preserved) with a German collection on *K. blossfeldiana* and to retrieve an ex-neotype reference sequence. A corresponding neotype has been designated and morphologically as well as phylogenetically examined. The morphology of the neotype material coincided with the asexual morph of *E. sedi* on *Sedum* spp. and the ex-neotype ITS1-5.8S rRNA-ITS2-28S rRNA sequence shows a congruence of 100% with sequences retrieved from *E. sedi* on *Sedum* and *Kalanchoe* spp. The results of a combination of morphological and phylogenetic analyses justify reducing *P. kalanchoes* to synonymy with *E. sedi*, while the limited resolution of ITS data on species level within the *E. aquilegiae* clade (see Takamatsu & al. 2015) may not be concealed. Multilocus analyses of this clade are necessary but not yet available.

TABLE 1. Host range and distribution of *Erysiphe sedi* on *Kalanchoes* spp.

Host	Country	Reference
Crassula ovata	Germany	Braun & al. 2003
Kalanchoe blossfeldiana	South Korea; Australia; Germany, Hungary, Norway, Romania, Switzerland; USA	von Arx 1952, Nagy 1975, Spencer 1978, Amano 1986, Ialongo 1992, Braun 1995, Jage & al. 2010
Kalanchoe daigremontiana; ≡ *Bryophyllum daigremontianum*	China, Nepal	Liu 2010 Adhikari 2017
Kalanchoe delagoensis; ≡ *Bryophyllum delagoense;* = *Kalanchoe tubiflora*	Germany; New Zealand	Braun & al. 2003; Boesewinkel 1980, as "*Microsphaera polonica*"
Kalanchoe pinnata; ≡ *Bryophyllum pinnatum*	China	Tang & al. 2016
Kalanchoe spp. [incl. hybrids]	Italy, Netherlands; Canada	Amano 1986
Sedum praealtum	New Zealand	Boesewinkel 1980, as "*Microsphaera polonica*"

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